

EUROFLEETS Cruise Summary Report

Project DIAPICNA

DIAzotrophic Plco-Cyanobacteria in the North Atlantic open ocean: their abundance and importance as a source of new nitrogen at the Azores Front/Current.

R/V NRP Dom Carlos I, Cruise No. DIAPICNA-OCE-2011-V01

25/07/2011 – 03/08/2011, Horta (Portugal) – Horta (Portugal)

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1 Summary

In the context of increasing atmospheric CO₂ concentrations, studies understanding and quantifying carbon fixation by oceanic biological production and its rate of removal to the deep ocean are of utmost importance to define the resilience of the Earth's biogeochemical cycles to anthropogenic forcing. Within the nitrogen biogeochemical cycle, Biological N₂ Fixation (BNF) is the only natural process which can relieve nitrogen limitation of atmospheric CO₂ sequestration. However, dissolved N₂ is available to only a small number of organisms (diazotrophs) producing the necessary enzymes for N₂ fixation and we are still at a rudimentary stage regarding our understanding of unicellular diazotrophs' distribution and contribution to oceanic BNF. The present research cruise aimed at unfolding the inter-linkages between N₂ and CO₂ fixation mediated by diazotrophs in an area of the North Atlantic Ocean where BNF is suspected to occur, but was never measured. The Azores Current region close to the Mid-Atlantic Ridge, an oligotrophic area where contrasting conditions are found at the interface between temperate and subtropical waters, was investigated for the presence, identity, distribution and activity of diazotrophs from the surface to deeper water layers. A South-North transect including 5 geographical sampling stations (Figs. 1 and 2) was performed to investigate the extent of BNF in subtropical/temperate waters. The focus was in particular on the possible impact of the Azores Front/Current system and of deep sea iron injection by the Rainbow hydrothermal system. The results are expected to contribute substantially to our understanding of the mechanisms leading to the development or limitation of BNF in the open ocean.

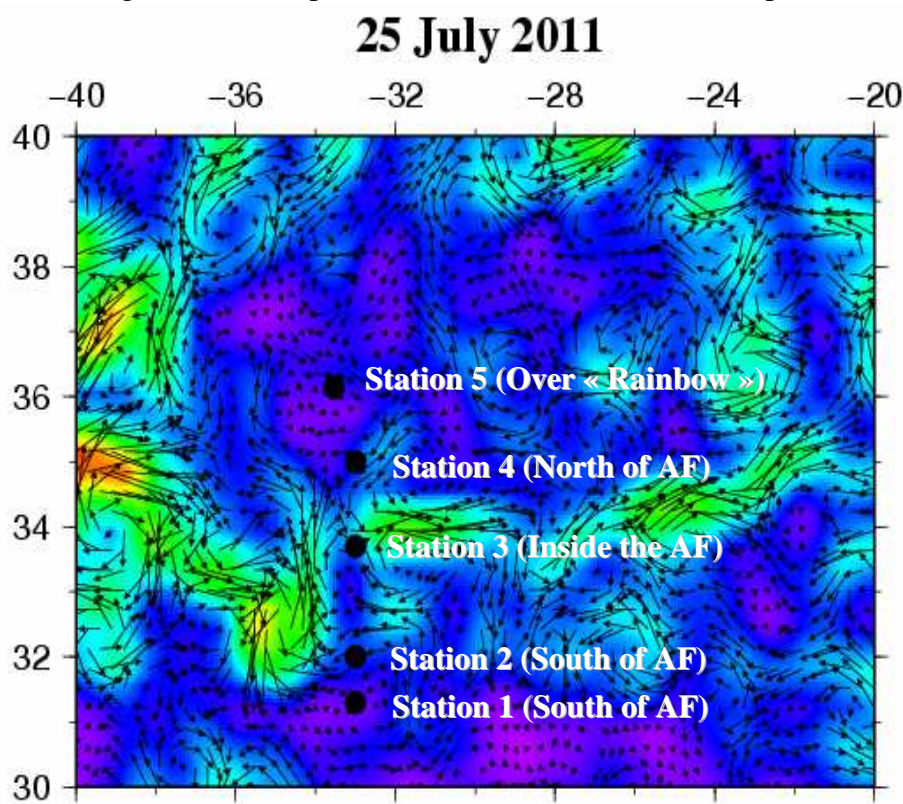


Fig. 1 Mean geostrophic currents obtained from AVISO Altimetry data for the study region at the time of DIAPICNA cruise departure (25th July 2011) aboard the R/V DOM CARLOS I. Black dots symbolize R/V DOM CARLOS I Cruise No. DIAPICNA-OCE-2011-V01 track chart. AF: Azores Front.

2 Research Programme/Objectives

The presence of the Azores Front/Current, a southern branch of the Gulf Stream penetrating to a water depth of at least 1000 m, about 50 km wide, forms a partial barrier to any ventilation of Eastern North Atlantic Water by southern water masses. North of the Azores Current (AC), the Azores Front (AF) separates the saltier and warmer southern 18°C Mode Water (18MW; a homogenous, well mixed water body which originates from the Sargasso Sea), from colder and fresher northern waters (i.e. the 15°C Mode water or 15MW). Important differences in primary productivity were measured between the 15MW and 18MW separated by the AF (Macedo *et al.*, 2000). N₂ fixation near the AF was estimated to account for up to 40 % of carbon export production, and to contribute relatively more to overall phytoplankton production south of the AF, compared to north of the front (Bourbonnais *et al.*, 2009). Surprisingly, no BNF activity or diazotroph diversity measurements have been published to date at the AF. The frontal zone sets the perfect basis for a natural *in situ* ecosystem-level experiment. Different physico-chemical conditions (Macedo *et al.*, 2000) and biological assemblages (Huskin *et al.*, 2004) are expected at either side of the front, which should enable testing the effects of temperature, O₂, NO₃⁻, NH₄⁺, PO₄³⁻ concentrations on diversity, abundance and activity of diazotrophs.

Sampling depth, water temperature, dark and light cycles, nutrient concentrations (PO₄³⁻, NO₃⁻, Fe) were indeed observed to impact the distribution of N₂ fixing organisms (Langlois *et al.*, 2008). The abundance of diazotrophs has been related to the supply of Fe-enriched dust (Langlois *et al.*, 2008), but the impact of deep sea hydrothermal iron injection has not yet been investigated. Although rapid progress is made, the contribution of individual diazotroph groups and the factors that constrain the growth of N₂ fixing microorganisms and N₂ fixation rates in the oceans are not well known. The North Atlantic Azores region benefits from Fe-rich dust inputs, as witnessed by the presence of such dust deposits (of probable Saharan origin) on the top of Pico volcano on the Azores island of Pico (PICO-NARE, Fialho *et al.*, 2006). In addition, the presence of the Rainbow hydrothermal vent of the Mid-Atlantic Ridge is a deep sea iron source close to the AF/C system (with a hydrothermal export flux of 9.6 mol Fe s⁻¹, German *et al.*, 2010). These different conditions and the technical/analytical means developed for the DIAPICNA cruise will hopefully yield exciting revelations on BNF in this part of the North Atlantic.

3 Narrative of the Cruise

The R/V *Dom Carlos I* arrived in Horta, Azores, on 23rd of July, 2011. Tenente Cardoso Jerônimo, a hydrographer detached by the Portuguese Hydrographical Institute (normally operating the R/V *Dom Carlos I* for hydrographical surveys) had made the voyage to Horta, to instruct the Oceanography team on the ship ADCP operation and the proper interactions between CTD operators, winch operators and bridge. These instructions were given during two days prior to the cruise departure (23th-24th July). The DIAPICNA cruise departed on the morning of July 25th after loading the last pieces of equipment. The ship stayed in the Pico-Faial Channel the first day of the cruise, allowing the physical oceanographers of the Universidade dos Açores (UAç) to practice and test the equipment under the supervision of the hydrographer. After validation of the procedures, the hydrographer was brought back on land, and at 8 p.m. local time R/V *Dom Carlos I* headed south to Station 1. The Portuguese navy hydrographer,

Lt. Américo Vidigal Alves, member of the R/V *Dom Carlos I* crew, now took over as liaison officer between the scientific team and the military crew.

The next 57 h of transit were entirely dedicated to the installation and testing of the complex sampling set-up of incubators and filtering devices. The latter were designed for size-fractionated CO₂/N₂-fixation measurements using stable isotope enrichments, detection of particulate organic carbon and nitrogen (POC/PN) natural isotopic composition, microscopic diazotrophic cyanobacteria outnumbering and identification, and RNA sampling for the relative activity determination of the different types of diazotrophic micro-organisms. During the transit time to station 1 we redefined the exact coordinates of stations 2, 3 and 4 according to the latest altimetry data (recorded July 25th) indicating the position of the AF. These latest positions and our related strategy were then communicated to Cpt. Moreira Pinto. The same period was used by Virginie Riou and Ana Martins to present the scientific goals of the cruise to the Portuguese navy crew.

As scheduled, the first station was sampled in the morning of 28th July and a second time later during the night (c.f. “7 Station List”). During the day an additional deep CTD cast was taken to better characterize the water column at our southernmost station, south of the Azores Front. Subsequently, the water column at stations 2, 3, 4 and 5 was sampled in the morning only. At Station 3 (intended inside the front) a further deep CTD was taken to confirm the Frontal structure. Stations 4 and 5 were also sampled for deep seawater (1900 m) because of their significance as a control site and a site under Rainbow hydrothermal influence, respectively. To locate precisely the site under influence of the Rainbow hydrothermal plume at Station 5, a strategy was developed by Virginie Riou and Lt. Américo Vidigal Alves, consisting in a transect using the multi-beam to record the precise bathymetry of the area, followed by an ADCP transect to determine the current direction at the depth of the plume dispersion (around 2000 m). Although the first deep CTD did not reveal any major turbidity anomaly (due to currents shift while cruise scanning), the next casts (down to 200 m) were performed at the defined position. A final deep cast at this position (initially dedicated to cable maintenance) revealed a turbidity anomaly around 2000 m. Water samples at 2000m were taken and the red-brown coloration of the filters showed presence of iron oxides, suggesting that we indeed sampled a dilute hydrothermal plume. Highly satisfied by this exceptional sampling, the whole team headed north to Faial on 1st August, and arrived at Horta on 3rd August.

4 Preliminary Results

4.1 Physical data (CTD and ADCP profiles)

(A. Martins, P. Shree Ram, V. Riou, F. Dehairs)

The initial positions of the five stations (determined prior to the ship departure, according to the position of the AF) shifted during the cruise as new AVISO altimetry data were being received (Fig. 1). At some point, the vessel was too far away from the coast and we no longer were able to access the internet and thus AVISO data. Therefore, only upon ship arrival on land (on the 3rd August, 2011), were we able to obtain new AVISO maps for the exact period of the cruise (Fig. 2). This new information suggests that Station 3 might have been positioned slightly south of the Azores Front and that Station 5 was positioned in the edge of an anticyclonic eddy (probably a Mediterranean Water Eddy, or MEDDIE).

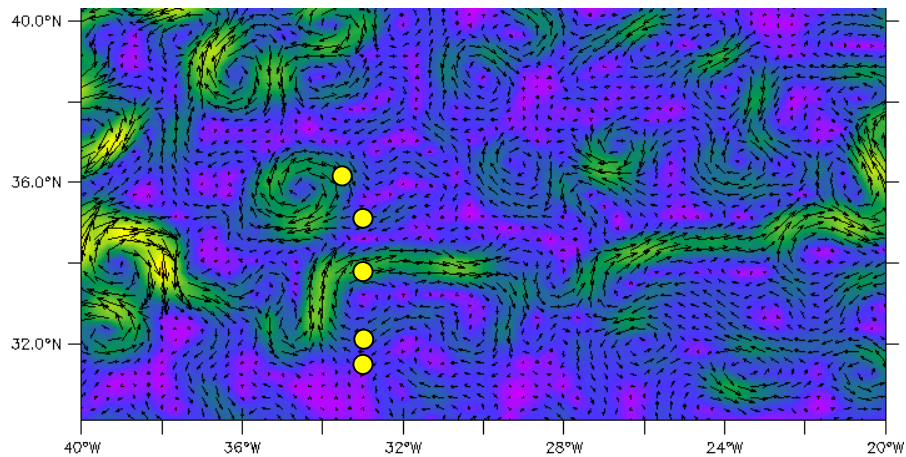


Fig. 2 Mean geostrophic currents obtained from AVISO Altimetry data for the study region for 1st August 2011 (day of station 5 sampling). Yellow dots symbolize R/V DOM CARLOS I Cruise No. DIAPICNA-OCE-2011-V01 track chart. AF: Azores Front.

While cruising, the vessel mounted ADCP (RDI Ocean Surveyor 38/150 kHz) was used to collect the ocean current measurements along the vertical column of the cruise track (e.g. Fig. 3). With a nominal profiling range of over 1000 m (in Long Range Mode, over 700 m in High Precision Mode), the 38 kHz Ocean Surveyor is an excellent frequency to ply the deep ocean. Therefore, for most observations we used narrow band 38.4 kHz to obtain the maximum vertical levels possible. Each vertical column has approximately 200 bins and each bin is spread about 40 m deep.

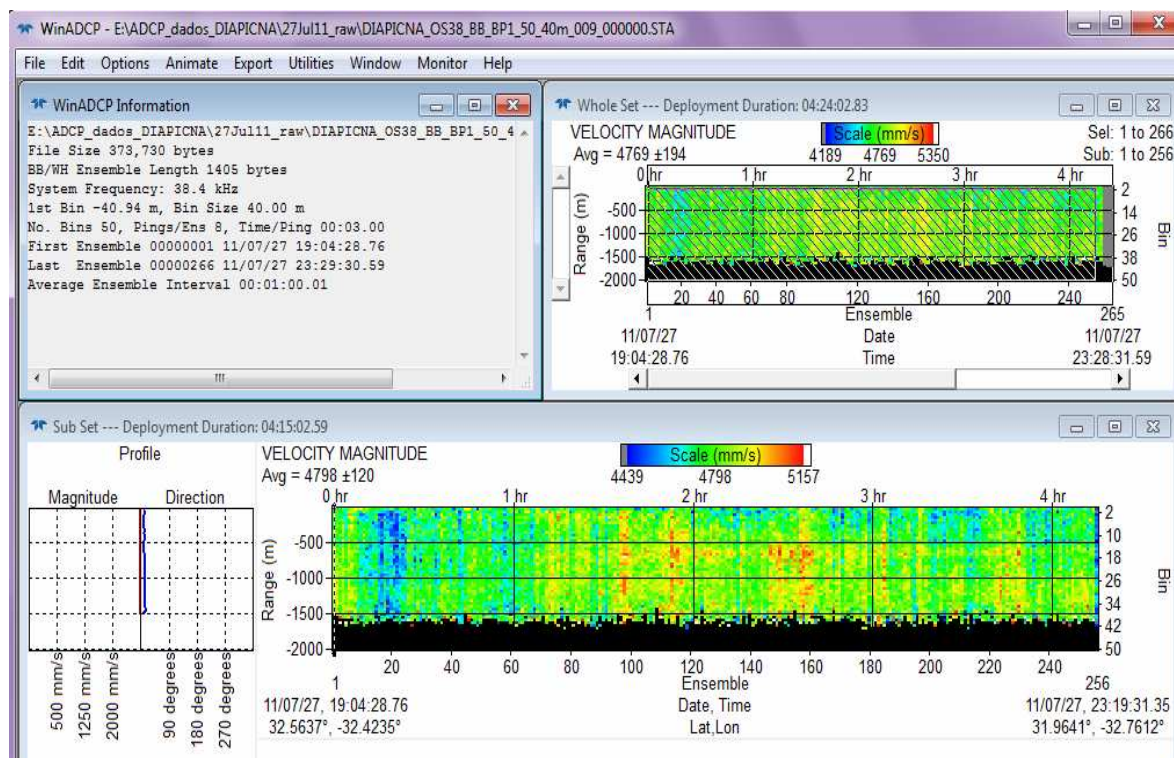


Fig. 3 Magnitude velocity as measured by the 38 kHz Ocean Surveyor while steaming at approximately 10 kts south of the Azores Current. x axis: ensemble number, y axis: range in meters; velocity range is indicated by the color bar at the top of the plot. Screen shot of the ADCP observations obtained on 27/07/2011 between 7.00 p.m. and 11.20 p.m.

The 38/150 kHz Ocean Surveyor was run via VMDAS under Windows. This program controls input and output data streams. The Ocean Surveyor performed extremely well during the cruise. The water profiling range hovered around 1500 m, depending on the presence of scatters in the water column. At each level, current direction, magnitude, u-component, v-component and vertical velocity were measured. ADCP was used between stations and switched off whenever other instruments like the Multibeam SIMRAD EM120; the Single beam Echo sounder 15/33/210 kHz or side scan sonar were in operation because this would interfere severely with the ADCP performance. ADCP data is currently under processing at DOP.

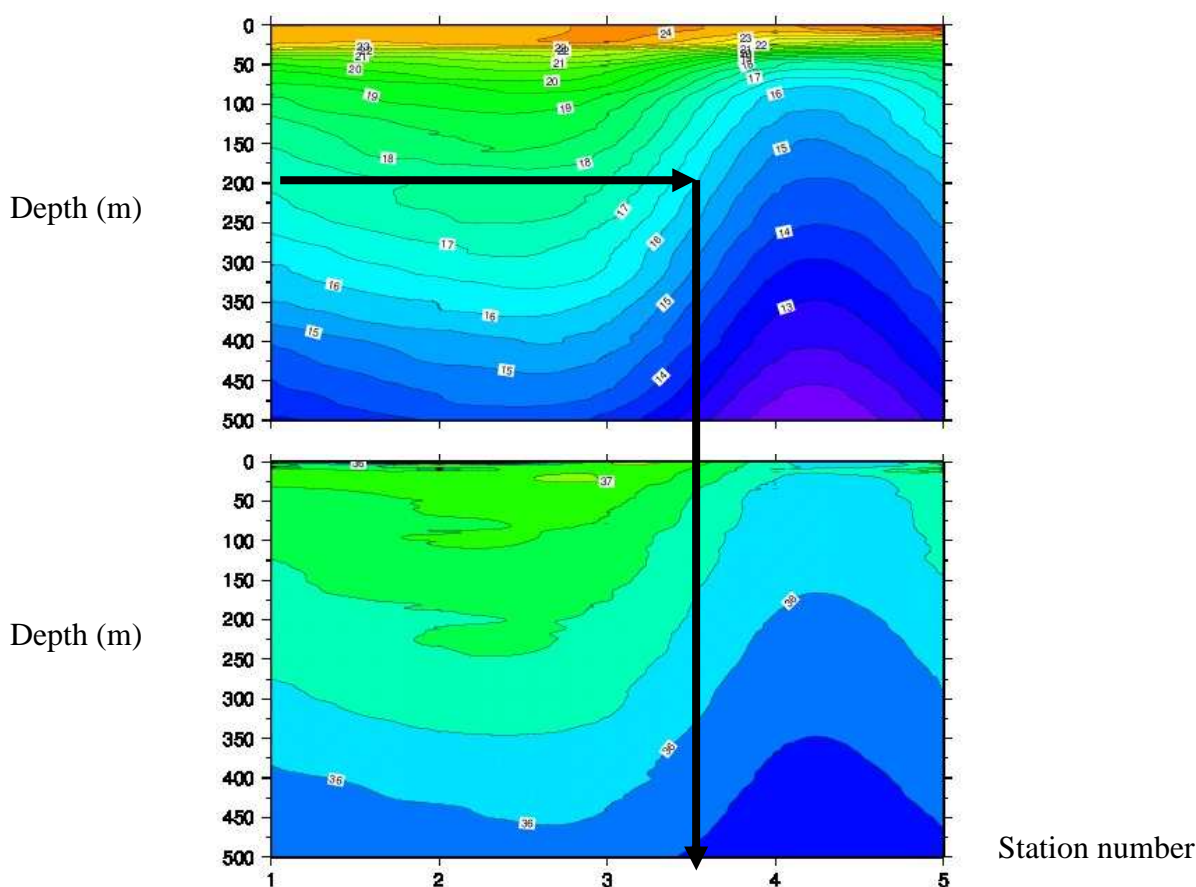


Figure 4 Vertical section of the temperature (°C, up) and salinity (psu, down) profiles measured by the SBE9plus probe in the euphotic layer along the sampling stations 1-5 (see 7 Station list for more information). Black arrow: position of the front according to Gould (1985).

A total of 35 casts were performed along the 5 stations (see § 7 Station List). The casts included the physical parameters temperature, conductivity (CTD), Photosynthetic Available Radiation (PAR), turbidity, oxygen and fluorescence profile measurements. The related *in situ* probes (SBE9plus, SBE19, SBE911, SBE43, and ECO FLRTD-1398, respectively) were incorporated into the SeaBird Rosette water sampler. These data are currently under analyses. Preliminary results suggest that Station 3 was in fact located close to, but slightly south of the Azores Current, as suggested by the AVISO altimetry maps (Fig. 4, up). According to Gould (1985), the approximate position of the Front can be determined by the 16°C isotherm at a depth of 200 m. The 16°C isotherm is observed around 100m deep at Station 4, north of the front, and goes down to 350m at Station 2, south of the front. Macedo et al. (2000) noticed that the frontal zone could also be detected by the deepening of the isohalines from the 15MW to the

18MW, which can not be detected at station 3 (Fig. 4 down). They also showed that the 15MW northern water mass is consistently less saline than the 18MW southern water mass at temperatures above 16°C, which we observe at stations 4 and 5 on the T-S diagram (Fig. 5).

A preliminary structure of water masses detected in the cruise survey region is presented in Fig. 5. Three distinct water masses are identified :

1- North Atlantic Central Water (NACW), with a linear decrease of T-S values from 100 m to about 600 m depth in the range of 24-18°C, and between 37-36 psu. This water is formed by subduction in polar fronts around Charlie-Gibbs and Maxwell Fracture Zones (FZ).

2- Mediterranean Water (MW) at depths of 600-1000 m. This 7-10°C cold water is detected by a peak in salinity with depth. O₂ minimum layers (not shown) were also observed at 600-1000 m in most casts, which could indicate Mixed Mediterranean Waters (MW).

3- Labrador Sea Water (LSW) at depths of 1500-2000m, detected by decreases in salinity and temperatures below 7°C.

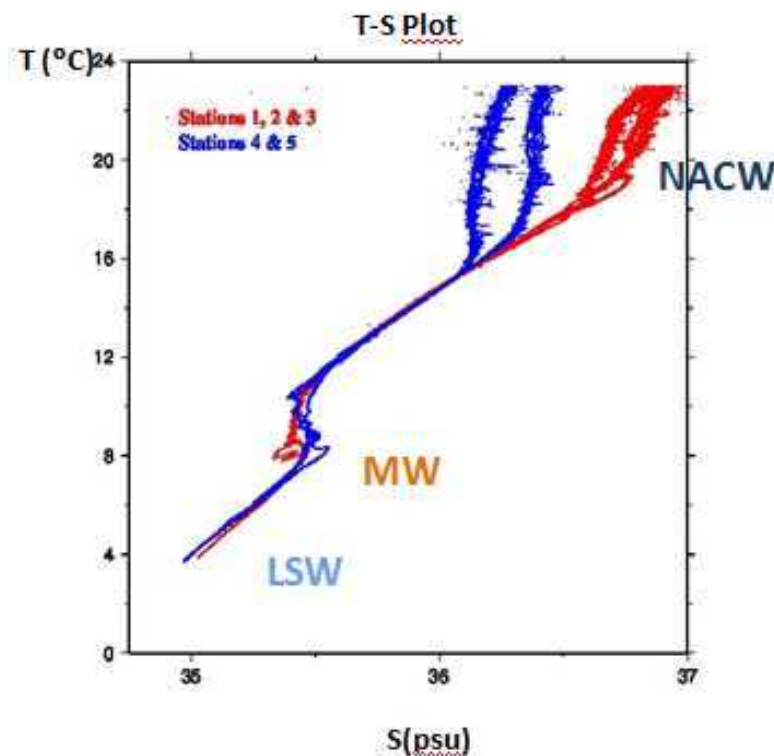


Fig. 5 Water mass characteristics of the cruise survey stations. T-S distributions. MW: Mediterranean Water; LW: Labrador Water; NACW: North Atlantic Central Water.

Satellite imagery of Ocean Colour (MODIS/Aqua) and Sea Surface Temperature (SST) (MODIS/Aqua and AATSR/ENVISAT) and altimetry (AVISO) are also currently under processing and analyses by the scientists from DOP to detect and track the AzC and AzF surface signatures (Martins *et al.*, 2004). Main satellite-derived near-surface chlorophyll *a* (in mg m⁻³) and surface temperatures (in °C) patterns will be studied for the cruise period and location.

4.2 Water and Plankton Sampling with CTD/Rosette

4.2.1 CTD Measurements and Sampling for nutrients and Stable Isotopes

(A. Martins, F. Dehairs and Shipboard Scientific Party)

Water was sampled with a SBE 32 Carousel Water sampler equipped with 12 2.5L Niskin bottles. Samples for O₂, nitrate + nitrite, ammonium, phosphate and silicate concentrations were taken at 12 depths in the upper 500 m. Nutrient analyses will be carried out both at VUB and UAç within the coming months, together with analysis of the nitrate N, O isotopic composition (at VUB; practical by master-2 student A. Roukaerts). The > 3 µm and 0.3-3 µm fractions of natural Particulate Organic Carbon/Particulate Nitrogen (POC/PN) were sampled at 5, 45 m, deep chlorophyll maximum (DCM) and 200 m depth, by filtering 4.5 L seawater over silver (3 µm) and glass fiber filters (0.3 µm). These samples are currently being analysed via Elemental Analyser-Isotope Ratio Mass Spectrometry (EA-IRMS) by master-2 student D. Batista at VUB for concentration and stable isotopic compositions.

4.2.2 Plankton sampling for identification

(V. Riou, C. Loureiro, M. Santos)

Chlorophyll *a* was sampled at every hydrocast/depth for concentration measurements (1L filtered on 47 mm GF-F filters, blotted dried, and stored dry, folded into tubes protected from sunlight, in liquid N₂). The pigments will be measured by the end of September at DOP/UAç by means of a spectrofluorometer, following the recommendations of Yentsch and Menzel (1963) and as described in Arístegui *et al.* (1997). For microplanktonic community composition and molecular analysis, duplicate 1 L samples of well mixed seawater were filtered under low vacuum pressure conditions onto 47 mm cellulose acetate filters (0.2 µm pore size) folded with flamed sterilized tweezers, and immediately frozen in liquid N₂ into sterilized tubes. Water samples were taken for phytoplankton microscopic taxonomic identification and quantification (5 mL preserved in formaline) and coccolithophore quantification (3L filtered on 47 mm 0.45µm porosity cellulose nitrate membranes, preserved in a dry place without light). Samples were specifically taken for diazotroph identification and quantification in Marseille: filtrations on 47 mm polycarbonate membranes (10, 3 and 0.2 µm pore size) and preservation for FISH (according to Biegala *et al.*, 2003) were performed on-board and will be analysed during D. Batista's master-2 practical. In addition, the link between activity and identity will be examined by quantifying specific *nifH* (gene encoding part of the protein catalysing N₂-fixation) mRNA from samples collected on 25 mm 0.2 µm Supor filters and preserved in *RNAlater* (these analyses will be performed within the year by V. Riou).

4.2.3 ¹⁵N-¹³C incubations for N₂-HCO₃⁻ fixation rates measurements

(F. Dehairs, D. Batista, A. Roukaerts, V. Riou)

A new stable isotope incubation method was used on board for BNF measurement (following Mohr *et al.*, 2010) and which was implemented as part of the master-1 practicals carried out by D. Batista (Poster, UPMC, Paris, June 2011), with the help of A. Roukaerts and under the supervision of F. Dehairs and V. Riou. Duplicate samples (4.5 L Nalgene

polycarbonate bottles), were taken at 5, 45 m, deep chlorophyll maximum (DCM) and 200 m depth, spiked with $^{15}\text{N}_2$ and $\text{H}^{13}\text{CO}_3^-$ and incubated for 24 h in on-deck incubators which were wrapped in neutral density blue screens mimicking in-situ PAR conditions. Incubators were kept at close to *in situ* temperature conditions with a continuous flow of surface seawater. At the end of the incubation period, subsamples were taken for assessment of the isotopic composition of DIC, N_2 . Then samples were filtered for collecting the suspended cells and particles. The $> 3 \mu\text{m}$ and $0.3\text{--}3 \mu\text{m}$ fractions were separated by filtration on silver and glass fiber filters, respectively. This will enable us to assess the importance of picoplankton BNF (mainly by unicellular diazotrophic cyanobacteria UCYN) compared to micro-/nano-plankton. The filters (and dissolved gas samples) are currently being analysed at VUB by D. Batista, using EA-IRMS.

5 Data and Sample Storage / Availability

Analyses at VUB and DOP will be finalised during the coming months. Once cross-checked and validated, results will be interpreted and published in international peer reviewed journals. Meta data will be made available to data banks in Belgium (Flanders Marine Research Institute, VLIZ a national oceanographic data centre and a Seadatanet partner) and incorporated to the Azores Oceanographic Data Centre (AZODC, <http://ocean.horta.uac.pt/azodc/>) created by DOP.

6 Participants

No.	Name	Gender	Affiliation	On-board tasks
1	Virginie Riou	F	VUB	RNA/FISH sampling, coordination
2	Frank Dehairs	M	VUB	^{15}N - ^{13}C incubations, nutrient sampling
3	Ana Martins	F	DOP/UAç	CTD-Rosette manipulation, CTD and ADCP analysis, coordination
4	Prakya Shree Ram	M	DOP/UAç	CTD-Rosette manipulation, CTD and ADCP analysis
5	Debany Fonseca Pereira Batista M.Sc. student	M	VUB	^{15}N - ^{13}C incubations, nutrient sampling
6	Arnout Roukaerts M.Sc. student	M	VUB	POC/PN and nutrient sampling
7	Clara Loureiro	F	DOP/UAç	CTD-Rosette manipulation, DNA, Chlorophyll sampling
8	Mariana Santos	F	DOP/UAç	CTD-Rosette manipulation, Nutrients, Plankton taxonomy and coccolithophores sampling
9	Sérgio Gomes	M	UAç	CTD-Rosette manipulation
10	Alexandre Medeiros	M	UAç	CTD-Rosette manipulation

VUB: Vrije Universiteit Brussel, Brussels, Belgium

DOP/UAç: Departamento de Oceanografia e Pescas/Universidade dos Açores, Horta, Portugal

7 Stations List

Station No.	Date	Time	Latitude	Longitude	Bottom Depth	Gear	Remarks/Recovery
	2011	[UTC]	[°N]	[°W]	[m]		
Faial	25.7.	14:54	38,46633	28,60660	616	ROS/CTD	Tests with expert from IH
Faial 2	25.7.	15:48	38,46658	28,60805	642	ROS/CTD	Tests with expert from IH
1	28.7.	07:04	31,49647	33,00310	4353	ROS/CTD	Profile/500-350-250-200-175-150-125-100-75-45-25-5 m
		09:55	31,49815	33,00037	4359	ROS/CTD	5 m 12 bottles
		10:50	31,49808	32,99957	4360	ROS/CTD	45 m 12 bottles
		11:28	31,49832	33,00145	4362	ROS/CTD	DCM/85 m 12 bottles
		12:20	31,49990	33,00267	4362	ROS/CTD	200 m 12 bottles
		14:07	31,49875	33,00058	4356	ROS/CTD	Deep cast 2000 m (only CTD profile)
		21:25	31,50102	33,00128	4356	ROS/CTD	Night Profile/500-350-250-200-175-150-125-100-75-45-25-5 m
		22:01	31,50000	33,00112	4353	ROS/CTD	Night/5 m 12 bottles
		23:30	31,49977	33,00150	4354	ROS/CTD	Night/45 m 12 bottles
		00:18	31,49860	33,00115	4358	ROS/CTD	DCM Night/90 m 12 bottles
		00:58	31,49893	33,00173	4355	ROS/CTD	Night/200 m 12 bottles
2	29.7.	08:27	32,20030	33,00197	3733	ROS/CTD	Profile/500-350-250-200-175-150-125-100-75-45-25-5 m
		09:46	32,20025	32,99992	3800	ROS/CTD	5 m 12 bottles
		10:33	32,19967	33,00043	3740	ROS/CTD	45 m 12 bottles
		11:28	32,19925	32,99985	3767	ROS/CTD	DCM/110 m 12 bottles
		13:17	32,19817	32,99992	3776	ROS/CTD	200 m 12 bottles
3	30.7.	09:43	33,70055	32,99940	3324	ROS/CTD	Profile/500-350-250-200-175-150-125-100-75-45-25-5 m
		11:02	33,70088	32,99693	3324	ROS/CTD	5 m 12 bottles
		11:33	33,70172	32,99883	3323	ROS/CTD	45 m 12 bottles
		12:58	33,69948	33,00283	3323	ROS/CTD	DCM/90 m 12 bottles
		13:54	33,69990	33,00298	3318	ROS/CTD	200 m 12 bottles
		14:30	33,69990	33,00320	3318	ROS/CTD	Deep CTD profile 1500 m
4	31.7.	06:00	35,00312	33,00312	3090	ROS/CTD	Deep cast 1900 m 12 bottles
		08:00	35,00027	33,00072	3091	ROS/CTD	Profile/500-350-250-200-175-150-125-100-75-45-25-5 m
		09:25	34,99973	32,99953	3092	ROS/CTD	5 m 12 bottles
		10:03	34,99987	32,00013	3091	ROS/CTD	45 m 12 bottles
		10:33	35,00045	33,00028	3091	ROS/CTD	DCM/80 m 12 bottles
5	01.8.	11:14	34,99993	32,99877	3091	ROS/CTD	200 m 12 bottles
		07:25	36,23143	33,89858	2272	ROS/CTD	Deep cast 1900 m 12 bottles
		09:21	36,23497	33,89957	2320	ROS/CTD	Profile/500-350-250-200-175-150-125-100-75-45-25-5 m
		10:42	36,23268	33,89885	2264	ROS/CTD	5 m 12 bottles
		11:28	36,23545	33,90253	2423	ROS/CTD	45 m 12 bottles
		13:12	36,23405	33,90003	2293	ROS/CTD	DCM/100 m 12 bottles
		14:04	36,23273	33,90135	2322	ROS/CTD	200 m 12 bottles
		14:58	36,23303	33,90083	2334	ROS/CTD	Deepn cast 2000m (5 bottles), 1410m (2 bottles), 910m (2 bottles), 80m (2 bottles) and 15m (1 bottle)

8 Acknowledgements

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